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EFFECTS OF ECTOPARASITISM ON BEHAVIORAL THERMOREGULATION IN THE TROPICAL LIZARDS *ANOLIS CYBOTES* (SQUAMATA: DACTYLOIDAE) AND *ANOLIS ARMOURI* (SQUAMATA: DACTYLOIDAE)

ASA E. CONOVER,[1,2] ELLEE G. COOK,[3] KATHERINE E. BORONOW,[4,5] AND MARTHA M. MUÑOZ[6]

ABSTRACT. A febrile response, or a raised body temperature in response to infection, has been widely documented in various species of reptiles in laboratory trials. However, whether and how behavioral fever is achieved in nature remains almost entirely unknown. Here, we examine whether two species of lizard in the cybotoid clade of Hispaniolan trunk-ground anoles (*Anolis cybotes* and *Anolis armouri*) change their basking behavior in response to infestation by the chigger mite, *Eutrombicula alfreddugesi*. We examined body temperature and basking behavior in wild populations of *A. cybotes* and *A. armouri* from four localities that spanned a 2,000-m elevational transect in the Sierra de Baoruco, Dominican Republic. Although basking rate increased with elevation, we found that it did not correlate with mite load. Body temperature was also unrelated to parasite load. Thus, we found that *E. alfreddugesi* infestation did not induce behavioral fever in these anoles. We found a strong altitudinal pattern in chigger infestations: Infestations levels were highest in lizards from mid-elevation and dropped dramatically at low and high elevation (particularly in the latter). We discuss possible mechanisms for this altitudinal pattern in chigger infestation and discuss the relationship between infection and behavioral thermoregulation in lizards.

KEY WORDS: behavioral fever; parasitism; lizard; chigger mites; behavioral thermoregulation; anole

[1] Stuyvesant High School, New York, New York, 10282, U.S.A.

[2] Department of Biological Sciences, University of Southern California, Los Angeles, California 90089, U.S.A.; e-mail: aeconove@usc.edu

[3] Division of Biological Sciences, University of Missouri, Columbia, Columbia, Missouri 65211, U.S.A.; e-mail: egcrg7@mail.missouri.edu

[4] Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138, U.S.A.; e-mail: kboronow@fas.harvard.edu

[5] Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138, U.S.A.

[6] Ecology, Evolution, and Genetics, Australian National University, Acton, Australian Capital Territory, Australia 0200; e-mail: martha.munoz@anu.edu.au.

INTRODUCTION

Many lizards respond to pathogen infection through a febrile response, or a marked increase in body temperature, which is proposed to enhance the inflammatory reaction (Vaughn *et al.*, 1974; Bernheim and Kluger, 1976; Bernheim *et al.*, 1978). Unlike endothermic animals such as birds and mammals, ectotherms rely heavily on thermoregulation to induce heightened body temperatures (Huey, 1982; Angilletta, 2009). Laboratory studies on lizards (Bernheim and

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Kluger, 1976; Muchlinski *et al.*, 1989; Ortega *et al.*, 1991; Ramos *et al.*, 1993; Scholnick *et al.*, 2010), crocodilians (Lang, 1987; Merchant *et al.*, 2007), turtles (Monagas and Gatten, 1983; Amoral *et al.*, 2002), and snakes (Burns *et al.*, 1996) have found that individuals injected with bacteria exhibit elevated set-point body temperatures, meaning that they attain warmer temperatures than uninfected individuals when placed in a temperature gradient and allowed to choose where to sit.

The laboratory studies discussed above suggest that behavioral thermoregulation mechanistically underlies fever, but this remains unconfirmed in wild populations of naturally infected lizards. In a semi-natural experiment, Muchlinski *et al.* (1989) found that, when injected with *Aeromonas* bacteria, free-ranging chuckwallas (*Sauromalus obesus*) exhibit heightened body temperatures, supporting the hypothesis that shifts in basking behavior are important for inducing fever. Malvin and Kluger (1979) found that iguanas do not increase their internal heat production to raise their core temperature during infection, further supporting the idea that extrinsic behavioral shifts are critical to achieving fever in lizards.

Here we tested whether infestations by chigger mites induced behavioral fever in anoles from the Dominican Republic. Ectoparasites such as chigger mites are known to affect lizard metabolism negatively (Booth *et al.*, 1993) and induce immune responses by skin inflammation (Goldberg and Bursey, 1991). Previous studies in lizards have found that ectoparasite infestation is associated with reduced body condition (Dunlap and Mathies, 1993; Klukowski and Nelson, 2001; Cook *et al.*, 2013), and reduced sprint speed (Main and Bull, 2000). In a previous study of *Anolis brevirostris*, an anole from the Caribbean island of Hispaniola, Cook *et al.* (2013) found that individuals with greater infestations of the chigger *Eutrombicula alfreddugesi* exhibited poorer body condition, had duller colored dewlaps (extensible throat fans), and displayed less frequently than individuals with lower parasite loads. Fence lizards with malaria also exhibit shifts in coloration (Ressel and Schall, 1989) and reduced body condition (Dunlap and Mathies, 1993), suggesting that ectoparasites have negative effects on their lizard hosts, either through the effects of the parasites themselves or through pathogens transmitted by the chiggers. Given the negative effects of chiggers, it is possible that lizards respond to infestation by these parasites through behavioral fever; by increasing their core temperature through increased basking, lizards may combat pathogens transmitted by the chiggers or cause the mites to drop off. The relationship between basking behavior and parasite infestation may further vary with altitude: In a study of three Hispaniolan anoles (*Anolis coelestinus*, *Anolis cybotes*, and *Anolis olssoni*), Zippel *et al.* (1996) found that chigger infestation levels increased dramatically with elevation. Given that basking frequency also tends to increase with elevation in some anoles from Hispaniola (Hertz and Huey, 1981; Muñoz *et al.*, 2014), the use of behavioral fever may be expected also to vary across altitude.

The goal of this study was to assess whether chigger infestations induce behavioral fever in wild populations of two species of *Anolis* lizards (*Anolis armouri* and *A. cybotes*) from the Dominican Republic, Hispaniola, arrayed across a 2,000-m elevational gradient. Specifically, we tested three hypotheses: (1) chigger infestation reduces lizard body condition; (2) lizards with greater parasite loads also have higher core body temperatures (i.e., exhibit a fever) and bask more than less infested lizards; (3) behavioral fever should be most pronounced at high elevation, where chigger infestations and basking rates are highest.

TABLE 1. SUMMARY DATA ARE GIVEN FOR EACH POPULATION FOR STUDY SITES IN THE SOUTHWESTERN REGION OF THE DOMINICAN REPUBLIC.

	Los Patos	Guayuyal	Polo	Zapoten
Coordinates	17° 57′ 36″N, 71° 11′ 24″W	18° 3′ 36″N, 71° 8′ 24″W	18° 10′ 12″N, 71° 15′ 0″W	18° 18′ 0″N, 71° 42′ 0″W
Elevation (m)	13	727	1,236	2,020
Mean annual temperature (°C)[a]	26.0	22.4	23.5	13.9
Sample size	18	18	20	22
Prevalence[b]	100	100	100	78.3
Infestation intensity[c]	29.4 ± 4.3	99.4 ± 18.4	108.4 ± 19.1	15.2 ± 4.4
Infestation range[d]	4–78	18–354	12–274	0–75
Observation time (min)[e]	58.2	57.7	59.1	57.2
Basking rate[f]	14.6 ± 1.0	44.4 ± 1.9	70.1 ± 2.1	90.3 ± 1.0
Body temperature (°C)[g]	30.9 ± 0.3	27.8 ± 1.7	26.7 ± 2.5	28.2 ± 2.3

[a]Mean annual temperature was extracted from the WorldClim database (Hijmans *et al.*, 2005).
[b]Percentage of individuals with at least one mite.
[c]Mean number of mites per individual (± 1 SEM).
[d]Range of infestation intensities for a population.
[e]Mean behavioral observation time.
[f]Percentage of time lizards were observed basking (± 1 SEM).
[g]Temperature ± 1 SEM.

METHODS AND MATERIALS

We conducted our study on the two cybotoid anoles, *A. cybotes* and *A. armouri,* in June and July 2012 in the Dominican Republic. The term "cybotoid" refers to the clade of anoles containing the widespread species *A. cybotes* and its relatives from the Caribbean island of Hispaniola (Glor *et al.*, 2003). Though *A. armouri* shares many overlapping morphological features with *A. cybotes* (Schwartz, 1989), this high-elevation specialist is considered a separate species (Glor *et al.*, 2003; Wollenberg *et al.*, 2013). Within the adaptive radiation of Caribbean anoles, the cybotoids all belong to the same "ecomorph" or habitat specialist category, meaning that they overlap substantially in behavioral, ecological, and morphological characteristics (Losos, 2009). Specifically, the cybotoids are "trunk-ground" anoles; as such, they perch close to the ground, especially on tree trunks, have stocky builds with long hindlimbs, and forage actively on the ground (Schwartz, 1989; Glor *et al.*, 2003; Losos, 2009).

We worked at four different localities ranging between 13 and 2,020 m in the Sierra de Baoruco in the southwestern region of the Dominican Republic (Table 1; Fig. 2). Study sites were located at Los Patos (13 m above sea level [masl]; 17° 57′ 36″N, 71° 11′ 24″W), Guayuyal (727 masl; 18° 3′ 36″N, 71° 8′ 24″W), Polo (1,236 masl; 18° 10′ 12″N, 71° 15′ 0″W), and Zapoten (2,020 masl, 18° 18′ 0″N, 71° 42′ 0″W). *Anolis cybotes* is found at the three low-elevation sites (Los Patos, Guayuyal, and Polo), where it occupies mesic, semi-disturbed habitats, especially near agricultural sites (plantain, coconut, and coffee plantations). The high-elevation specialist *A. armouri* is found in Zapoten. At this montane locality the habitat is composed of monodominant pine forest and open fields with rocky outcrops.

Eutrombiculid mites are known to parasitize anoles from low- to mid-elevation (~ 520 masl) on Hispaniola (Zippel *et al.*, 1996). These

Figure 1. A male *Anolis cybotes* infested with *Eutrombicula alfreddugesi* mites on its dewlap (throat fan).

small, orange-colored mites are most commonly found in densely vegetated areas with high humidity and moderate ambient temperature (Clopton and Gold, 1993; Bulté *et al.*, 2009). The larvae attach to anoles through direct contact and typically cluster in skin folds, especially behind the front and back limb joints and on the dewlap, an extensible throat fan used extensively in *Anolis* communication (Fig. 1). Mites can cause lesions, blood loss, and skin inflammation in lizards (Goldberg and Bursey, 1991; Goldberg and Holshuh, 1992) and can transmit pathogens. In Asia, chigger mites are known to transmit scrub typhus (Traub and Wisseman, 1974), and, more generally, mites might be vectors for bloodborne parasites known as haemogregarines, which are known to reduce burst speed in lizards (Oppliger *et al.*, 1996; Garrido and Pérez-Mellado, 2014).

Following Rand (1962), we found focal lizards through slow, random sweeps through the habitat over the course of 2–3 days per site during all weather conditions except rain. All observations were made on undisturbed, naturally behaving adult male animals following the methods of Johnson *et al.* (2010). We performed behavioral observations either by watching lizards with binoculars and manually transcribing observations or with video recordings (Sony HDR-XR500V camera, set in standard definition), which we subsequently scored. Observations lasted from 30 to 60 min ($\bar{X}$ = 57 min) per lizard.

During each behavioral observation period we recorded the amount of time the lizard spent under different basking conditions. We recorded each lizard's basking status, which refers to how it was exploiting weather conditions, following Muñoz *et al.* (2014). During each observation period we recorded the weather conditions as sunny, partly sunny, or overcast. Under sunny or partly sunny conditions lizards could be scored as perching in the sun, in the partial sun, or in the full shade. Under overcast conditions lizards could only be in the shade (i.e., there was no basking choice), and these observations were discarded from further analysis. At the end of the observation, the relative amount of time each lizard spent basking was calculated as the total time spent either in the full or partial sun divided by the length of the total observation.

At the conclusion of each observation we attempted to catch the lizard to obtain information on body temperature, body condition, and parasite infestation. We noosed lizards using a dental floss noose tied to the end of a 10–12-foot (3–3.7 m) telescopic panfish pole (Cabela's Incorporated, Sidney, Nebraska). Immediately after capture, we measured the core temperature of the lizard by inserting a thermocouple (type T, copper-constantan; Omega Engineering) approximately 1 cm into the lizard's cloaca. The thermocouple was attached to a handheld

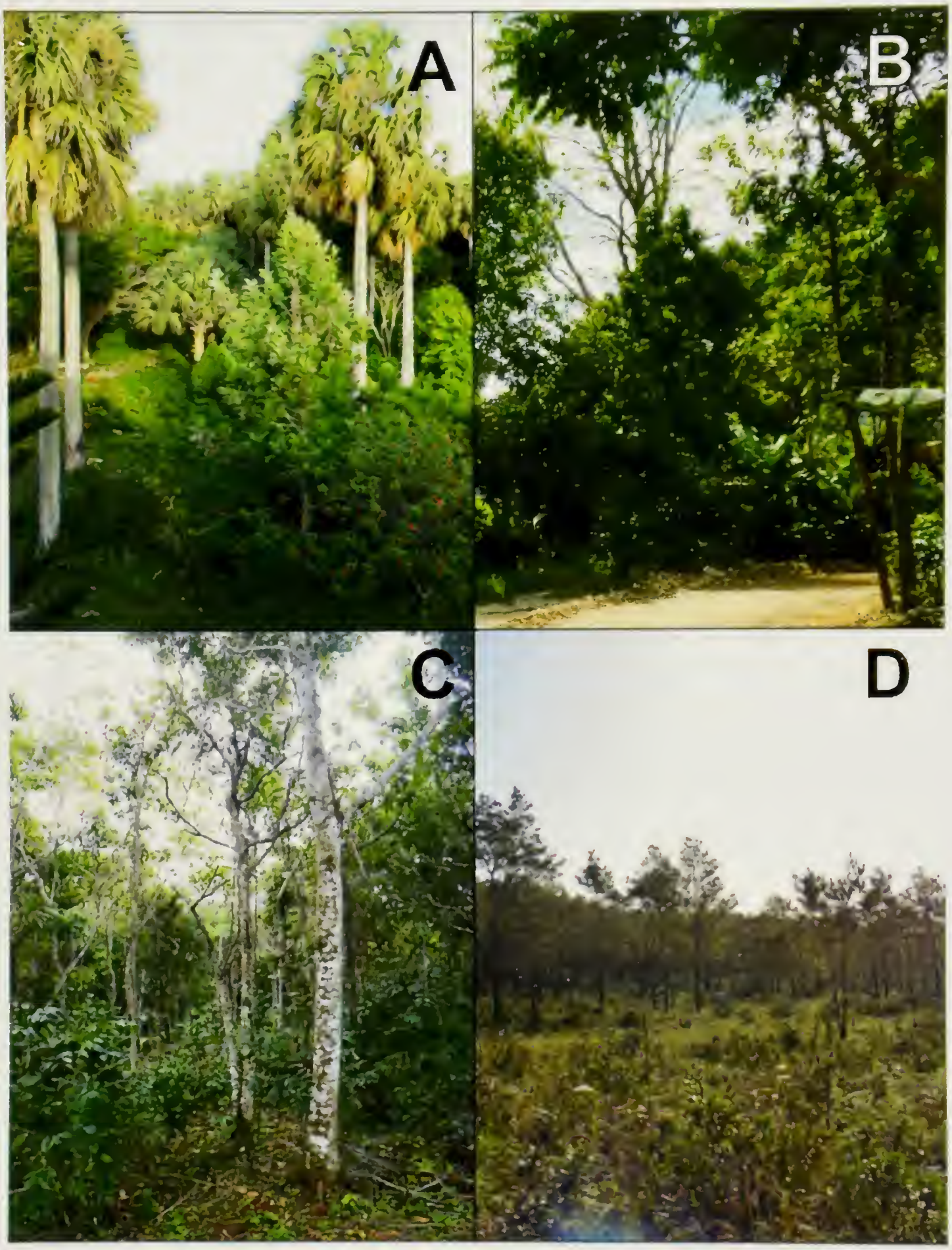

Figure 2. Images showing the four localities where this study was conducted: A, Los Patos (13 m elevation); B, Guayuyal (727 m); C, Polo (1,236 m); and D, Zapoten (2,020 m).

reader (model HH603A; Omega Engineering), which gave temperature measurements to the nearest 0.1°C.

For each lizard captured we also measured body mass to the nearest 0.1 g, using a spring scale, and body size as snout-vent length (SVL), the distance from the tip of the snout to the anterior edge of the cloaca, using digital calipers (Mitutoyo), which gave length measurements to the nearest 0.01 mm. We then counted the total number of ectoparasites on each anole using a handheld loupe. We sampled individuals only once and released them at the site of capture within 48 hours.

For each population, we calculated prevalence, which refers to the proportion of individuals in a population that had at least one mite. Following Margolis *et al.* (1982), we measured infestation intensity as the number of mites per lizard. We estimated body condition as the residuals of body mass regressed against SVL (Schulte-Hostedde *et al.*, 2005). Before statistical analyses, we log-transformed all continuous variables and arcsine square root–transformed the proportional variable (basking rate). Given that infestation intensity correlated strongly with body size (Pearson's $r = 0.441$, d.f. $= 76$, $p < 0.001$), we used the residuals of infestation intensity regressed against SVL as our measure of parasite load in our examinations of correlation with body temperature and basking rate. We assessed the Pearson correlation between variables, and used the Bonferroni correction to correct for multiple tests. None of the relationships were changed when we compared body temperature and basking rate to infestation intensity, rather than parasite load. We compared body temperature among populations using the Mann-Whitney *U* test.

RESULTS

Prevalence was extremely high across localities: 73 of the 78 lizards examined were infested with *E. alfreddugesi* mites, and 100% of lizards from the three lower elevation sites were infested (Table 1). Prevalence was slightly lower in Zapoten, where 21.7% of lizards captured had no parasites, and most individuals had fewer than 20 mites (Fig. 3). On average lizards harbored 61.7 mites, and infestation intensity ranged from 0 to 354 mites (Table 1). Despite high numbers of ectoparasites, we found no significant relationships between infestation intensity and lizard body condition (Table 2).

Basking behavior differed among localities (Table 1): The proportion of time lizards spent basking increased with elevation from 14.6% in Los Patos (13 m) to 90.1% in Zapoten (2,020 m). Mean body temperature ranged from 26.7°C (Polo) to 30.9°C (Los Patos) (Table 1) and was significantly higher in Los Patos relative to the other populations (Mann-Whitney *U* test; all $p < 0.01$). However, basking rate and parasite load were not strongly correlated (Table 2). Similarly, we found no significant relationships between body temperature and parasite load (Table 2).

DISCUSSION

Behavioral fever is one way that ectothermic animals might be able to respond to parasite infestation. However, we found that basking frequency and body temperature were not correlated with parasite load (Table 2), indicating that *A. cybotes* and *A. armouri* did not exhibit a febrile response to chigger infestation. One possible explanation is that anoles do not exhibit behavioral fevers. Muchlinski *et al.* (1995) found that *Anolis equestris* injected with bacteria did not exhibit elevated core temperatures compared with uninfected individuals. In another study, *Anolis carolinensis* that were injected with bacterial lipopolysaccharide exhibited a hypothermic response (i.e., cooler,

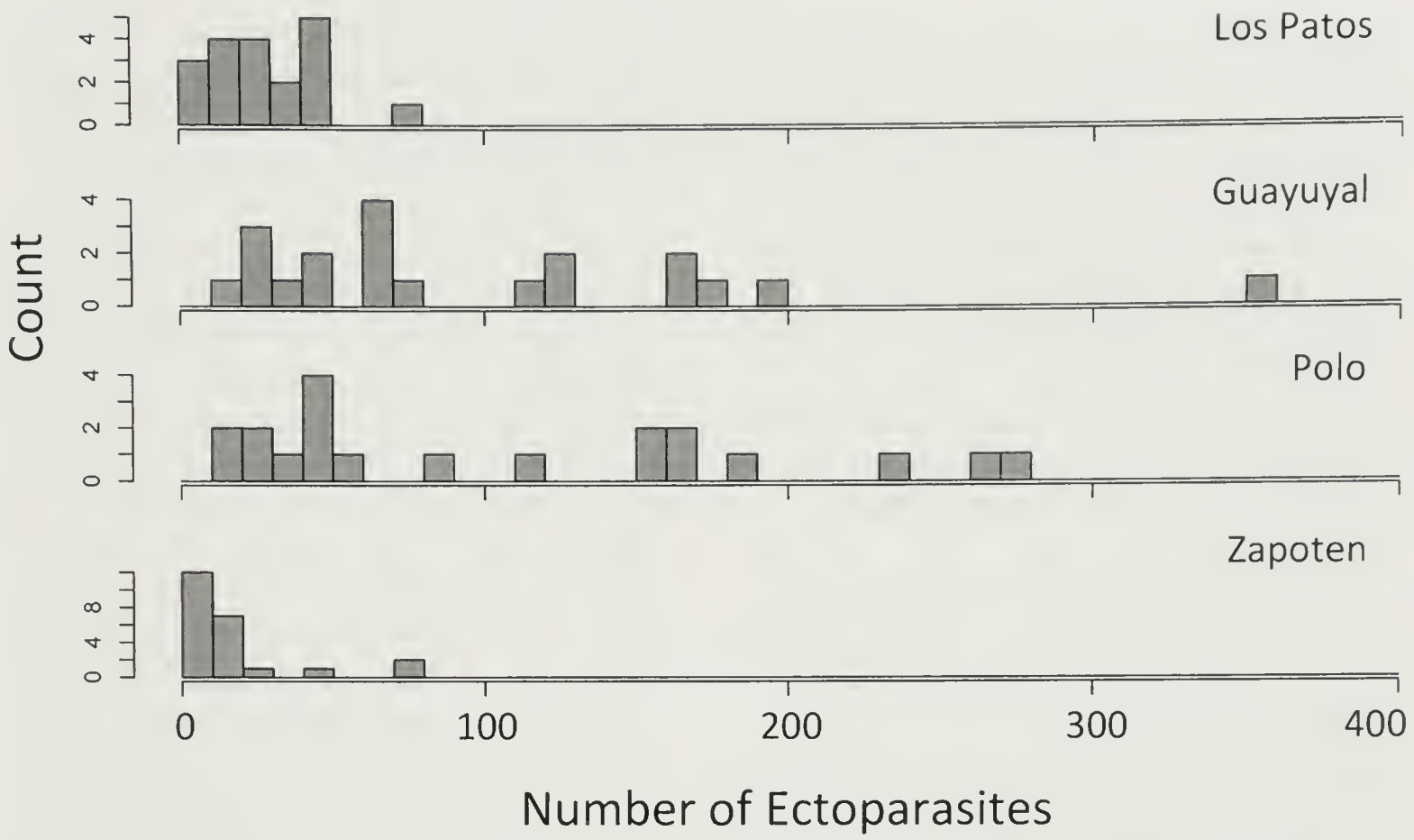

Figure 3. Histogram showing infestation intensity frequency in each of the populations sampled.

Table 2. Results for Correlation Tests Examining Relationships Between (A) Body Condition, (B) Basking Rate, and (C) Body Temperature and Parasite Load. Pearson's *r* and *P* Values are Given for Each Test, and Sample Size is Given in Parentheses.

	r	*P*
A. Body condition ~ infestation intensity[a]		
Los Patos (18)	−0.161	0.525
Guayuyal (18)	0.326	0.187
Polo (20)	0.231	0.328
Zapoten (22)	−0.319	0.148
B. Basking rate ~ parasite load[b]		
Los Patos	0.262	0.293
Guayuyal	−0.399	0.101
Polo	−0.129	0.599
Zapoten	0.033	0.896
C. Body temperature ~ parasite load		
Los Patos	−0.080	0.753
Guayuyal	−0.489	0.040
Polo	−0.219	0.353
Zapoten	0.036	0.875

[a]Residuals of body mass/SVL and the number of ectoparasites (infestation intensity). SVL refers to the body size of the lizard, measured as the distance from the tip of the snout to the cloaca (snout-vent length).

[b](Time spent basking)/(total observation length) and parasite load (residuals of infestation intensity ~ SVL).

rather than warmer, body temperatures) when placed in a temperature gradient and allowed to choose where to sit (Merchant *et al.*, 2008). Looking more broadly, whereas many studies have observed a febrile response to infection in lizards (e.g., Bernheim and Kluger, 1976; Muchlinski *et al.*, 1989; Ortega *et al.*, 1991; Ramos *et al.*, 1993), others have failed to detect a pattern (Laburn *et al.*, 1981; Mitchell *et al.*, 1990) or have found that individuals can vary in whether or not fever is induced (Bernheim and Kluger, 1976).

Behavioral fever might not be prevalent in lizards because the costs associated with fever are too high to induce them in nature. Almost all studies examining febrile responses in lizards were conducted using laboratory heat gradients, where all other ecological variables besides infection are held constant. Given that extra time spent thermoregulating imposes a cost to other activities, such as foraging, predator avoidance, and reproduction (e.g., Huey, 1974; Grant and Dunham, 1988; Adolph and Porter, 1993), it is possible that selection does not favor fevers in nature. It is also possible that the chigger mites can withstand more heat than *A. cybotes* and *A. armouri*. Tropical lizards such as anoles tend to exhibit low body temperatures and heat tolerances relative to other lizard species (discussed in Sunday *et al.*, 2010; Araújo *et al.*, 2013); it is possible that a febrile response is not effective for lizards with low heat tolerances, such as these species (critical thermal maximum: ~ 38–40°C; Muñoz *et al.*, 2014), although it may be possible and advantageous in more heat tolerant species.

It is also possible that mite infestations do not negatively affect lizards enough to induce fever in *A. cybotes* and *A. armouri*. Although some studies have found negative correlations between chigger infestation and lizard body condition (Dunlap and Mathies, 1993; Klukowski and Nelson, 2001; Cook *et al.*, 2013), we did not observe any correlation between body condition and chigger infestation (Table 2). Similarly, other studies have found no effect of *E. alfreddugesi* infestation on lizard health (García-De La Peña *et al.*, 2004, 2010; Schlaepfer, 2006; Rocha *et al.*, 2008), and others have found positive correlations (Amo *et al.*, 2005), suggesting that the health effects of mite infestation may vary among taxa. This variable response in host health is not limited to chiggers: Even when infected with malaria, some anole species exhibit negative effects, whereas others do not (Schall, 1992; Schall and Pearson, 2000). These findings underscore that a more comprehensive understanding of how ectoparasites (and the pathogens they may transmit) affect lizard health is necessary for determining whether behavioral fever should occur.

More broadly, it is still not fully understood how ectoparasite infestation should affect lizard body condition. For example, the detrimental effects of ectoparasites can be inferred from both negative correlations between body condition and mite loads (Dunlap and Mathies, 1993; Klukowski and Nelson, 2001; Cook *et al.*, 2013) and from positive correlations (e.g., Amo *et al.*, 2005). In the former, the reduced body condition of infested lizards can suggest that the ectoparasites reduce health and immunity, as evident in the reduced body condition, and in the latter it is thought that parasites reduce survival and so only the individuals with best body condition can survive (Amo *et al.*, 2005). Furthermore, even when negative correlations between body condition and parasite load are detected, they may not be driven by the parasites inducing lizards to lose mass. Klukowski and Nelson (2001) found that body condition was reduced in infested lizards because high mite loads appeared to prevent lizards from gaining

mass. In short, a deeper understanding of how ectoparasites and their associated bloodborne pathogens influence host health will vastly improve our predictions for how behavioral fever should occur, if at all.

Although basking rate was uncorrelated with parasite load, the use of sun and shade varied considerably across elevation. Whereas lizards near sea level spent only a small fraction of their time perching in the sun, those found at high elevation (Zapoten) were almost invariably observed basking (Table 1), a result that aligns with findings from previous studies on these species (Hertz and Huey, 1981; Muñoz *et al.*, 2014). Prevalence was also considerably lower at Zapoten, and most lizards harbored fewer than 20 parasites (Fig. 3). If increased basking frequency were associated with warmer core temperatures in Zapoten, then it could be possible that fever in *A. armouri* reduced ectoparasite levels. However, this is unlikely because mean body temperature was significantly lower in Zapoten ($\bar{X}$ = 28.2°C) than in Los Patos ($\bar{X}$ = 30.9°C), suggesting that even though they bask continually, lizards in high-elevation populations might not always be able to attain body temperatures comparable to their low-elevation counterparts, let alone behaviorally induce a fever.

The markedly low levels of infestation intensity observed in *A. armouri* from Zapoten appear to conflict with previous findings on *A. cybotes* by Zippel *et al.* (1996), who found that intensity increased with elevation. Both their study and ours were conducted in the Sierra de Baoruco mountain chain in the western Dominican Republic. However, Zippel and colleagues did not sample *A. cybotes* lizards above 520 m, and our transect extended to 2,020 m. Consistent with Zippel *et al.* (1996), we found that mite infestation increased from sea level to mid-elevation, as intensities were particularly high in the mid-elevation populations at Guayuyal (727 m) and Polo (1,236 m), where up to 354 and 274 ectoparasites were observed on a single lizard, respectively (Table 1; Fig. 3). The infestation levels in Guayuyal and Polo are among the highest recorded for mites on other species of lizards (Amo *et al.*, 2005; Rocha *et al.*, 2008; García-De La Peña *et al.*, 2010; Delfino *et al.*, 2011; Ramírez-Morales *et al.*, 2012; Cook *et al.*, 2013, but see García-De La Peña, 2011), as well as for lizards with other types of ectoparasites such as ticks (e.g., *Ixodes ricinus* [Acari: Ixodidae]: Amore *et al.*, 2007; Gryczyńska-Siemiątkowska *et al.*, 2007; Stuart-Fox *et al.*, 2009; Gomes *et al.*, 2013).

Why do intensities drop so dramatically in Zapoten? Habitat preference by *E. alfreddugesi* may explain particularly low infestation levels observed at high elevation. Previous work has shown that chigger mites tend to prefer mesic habitats with low-incident sunlight and moderate temperatures (Clopton and Gold, 1993; Schlaepfer and Gavin, 2001) and that parasite intensity is typically higher in forest interiors than in forest edges (Bulté *et al.*, 2009; Rubio and Simonetti, 2009). At elevations above approximately 1,800 m in Hispaniola, anoles tend to cluster in forest clearings and rocky outcrops, presumably to access open basking sites in this colder environment, where temperatures can reach near freezing throughout the year (Hertz and Huey, 1981; Muñoz *et al.*, 2014). Even contiguous forest tends to be more open than at low elevation because the pine forest lacks the closed canopy characteristic of the broadleaf forest at lower elevations (Fig. 2) (Martin *et al.*, 2011). Thus, open habitat and cold temperatures may prevent *E. alfreddugesi* from reaching densities comparable to those observed at lower elevations.

Although behavioral fever is likely a key response to infection in lizards, we still know little of how it occurs in nature. In the case of *A. cybotes* and *A. armouri*, we did not find evidence that lizards respond to ectoparasite infestation through behavioral fever. It is not fully known how these (and other)

ectoparasites influence their lizard hosts and what pathogens they transmit (Amo *et al.*, 2005; Garrido and Pérez-Mellado, 2014). A more detailed understanding of how ectoparasites and bloodborne pathogens influence lizard health and how pathogen and host temperature tolerances differ will lead to more detailed hypotheses about the conditions under which we expect lizards to exhibit behavioral fever.

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